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Rapid report

Higher cholesterol in human LDL is associated with the increase of oxidation susceptibility and the decrease of antioxidant defence: experimental and simulation data

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Abstract

Increased low-density lipoprotein (LDL) cholesterol is a recognized risk factor for atherosclerosis. There is also strong evidence that oxidatively modified LDL initiates the development of this pathological process and the administration of antioxidants might have a protective effect. However, the appropriate trials did not provide completely consistent results. We found in this study that the oxidation kinetics and also the antioxidant effectiveness are different depending on the cholesterol content in LDL. Higher cholesterol in LDL causes an acceleration of its oxidation as well as an increase of resistance to the antioxidative effect of ascorbic acid. In searching for a theoretical background of this dual impact of cholesterol in LDL, computer simulation of LDL oxidation was used. It was found that the pre-existing level of lipid hydroperoxides together with the total amount of oxidizable lipid substrate associated with the cholesterol level in LDL were satisfactory prerequisites for a best fit to the experimental data. In conclusion, this study provides at least a partial explanation for some failures to arrest, by administration of antioxidants, the progression of atherosclerosis in animal and human hypercholesterolemia.

Keywords: Low density lipoprotein; Oxidation susceptibility; Antioxidant defense

An increased concentration of plasma low-density lipoprotein (LDL) cholesterol constitutes a major risk factor for atherosclerosis and cardiovascular disease. Several lines of evidence support a role for oxidatively modified LDL in atherosclerosis and for its in vivo existence [1]. Antioxidants have been shown to decrease atherosclerotic lesion formation in some [2] but not in all animal models especially when cholesterol levels remained markedly elevated [3–7]. During our studies aimed at elucidating the relation between cholesterol and oxidation kinetics of LDL [8,9] we noticed that the same amount of antioxidant added to various LDL samples had variable impact on LDL oxidizability. This effect apparently correlated negatively with the content of LDL cholesterol. Therefore, we undertook a more detailed analysis because of possible linkage of this effect to the above mentioned discrepancies.

Isolation of LDL from human serum of volunteers and copper catalyzed oxidation was carried out as described earlier [9]. The oxidation of LDL was followed according to the absorbance change at 234 nm corresponding to conjugated dienes during 5–24 h (see [10]). The absorption was set to zero at the beginning of the reaction. The ascorbic acid was added immediately before oxidation of the LDL samples. The addition of ascorbic acid caused a prolongation in lag time of oxidation as expected [10,11] but to

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various extents (Fig. 1). The samples were therefore allocated to groups H, M and L. In group H the effect of ascorbic acid was very small or almost negligible, the related level of LDL cholesterol was 4.1 mmol l^{-1} and the initial lag time was 59.4 min. In group M the effect of ascorbic acid was intermediate, the LDL cholesterol was 3.2 mmol l^{-1} and the initial lag time was 84 min. In group L the lag time was most prolonged ending not before 7 h due to ascorbic acid, LDL cholesterol was 1.9 mmol l^{-1} and the initial lag time was 202.8 min. In all groups the protein content was close to 100 μg ml^{-1} of sample.

The basic reactions used by the simulation are described in Table 1. The regression analysis and analytical integration were carried out with a computer-aided program, Origin 4.1 (MicroCal Software) and Matlab 4 (The Math Works), respectively. The program used for numerical integration of differential equations related to the mass action kinetics of Table 1 was the GEPASI package of Windows 95, version 3.1 [12,13]. The average computing time for a full reaction set was less than 0.5 s for a real time of 8 h. This program was unique in providing reliable solutions to many problems involving differential equation sets.

The time courses of LDL oxidation in group H, M and L are depicted in Fig. 1.

Data (average from three experiments) marked by squares, diamonds and circles correspond to controls and 2.25 or 4.5 μmol l^{-1} concentration of ascorbic acid added to the LDL samples. One can see the remarkable difference in lag phase and antioxidant effectiveness between the different groups of LDL samples.

There is no straightforward explanation for the above mentioned phenomenon because the oxidation of LDL is relatively complex and a tightly ordered process even under in vitro conditions [14]. Nevertheless, the problem can be accessible with the help of some simulation models (e.g. [15,16]). Basic elementary reactions are listed in Table 1. Those numbered 1 and 2 can be solved analytically. It leads to the equation

$$\mathrm{LOOH} = \mathrm{LH}\times[\mathrm{LOOH}]_i/([\mathrm{LOOH}]_i - \exp(-k\times\mathrm{LH}\times\mathrm{time})\times[\mathrm{LOOH}]_i + \exp(-k\times\mathrm{LH}\times\mathrm{time})\times\mathrm{LH}),$$

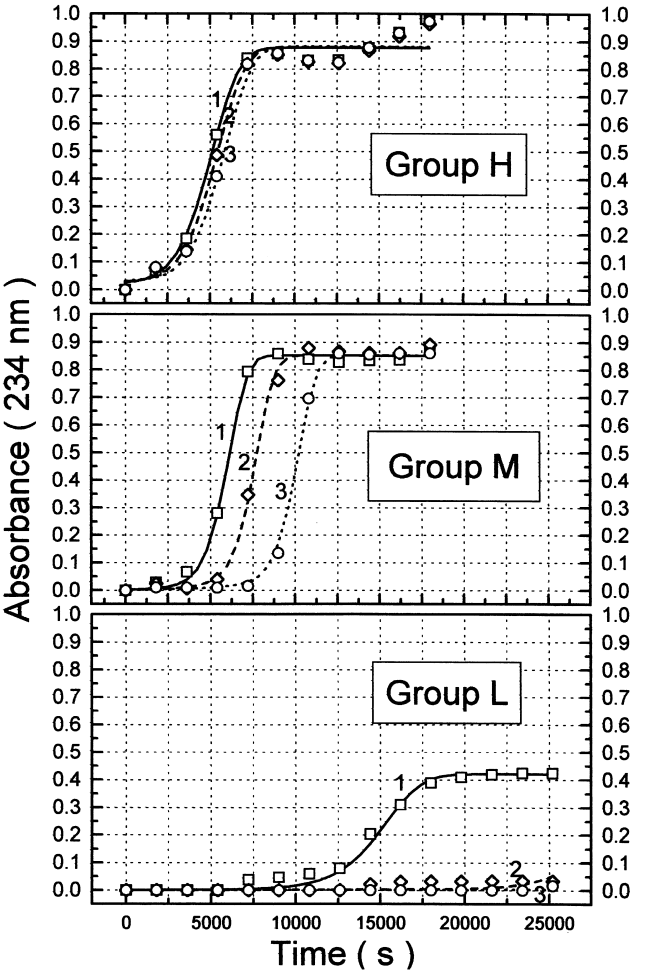

Fig. 1. The time course of oxidation of human LDL of various cholesterol contents and effect of ascorbic acid. Symbols represent the experimental data and the lines the simulation data. Data marked by squares and/or by line 1 correspond to the control, whereas data marked by diamonds, circles and/or lines 2 and 3 were obtained in the presence of increasing concentration of ascorbic acid. For more details, see the text.

where k is constant, $[\mathrm{LOOH}]_i$ is the initial concentration of LOOH (see also Table 1). The equation was used for the regression of experimental data that provided first estimates of maximum and initial values of LOOH in the groups given in Table 1.

Numerical integration of all reactions 1, 2, 3 and 4 expressed as a set of simultaneous differential equations of the first order proved to be more informative. The exploration of the best fit of the model to experimental data by scanning of 'parameters space' and experimentation with the model showed that the preformed level of lipid peroxides alone (see LOOH in reaction 1) was the main determinant of oxidation

susceptibility and effectiveness of antioxidant. This agrees with the experimental findings that the manipulation of the amount of preformed LOOH dramatically changes the lag time [8,17,18]. The length of the lag phase was linearly and negatively related to the logarithm of LOOH concentration. As a corollary of this finding, the effect of antioxidant increased exponentially with its dose but it was almost negligible when the level of preformed LOOH was high. The raising constant of reaction 2 influences positively the rate of the propagation phase. Reaction 3 describes a requirement of LDL oxidation for oxygen. The model agrees with the fact that the oxygen concentration in the laboratory is not rate limiting for LDL oxidation. It predicts, however, that increasing lack of oxygen might play an important role in the defence mechanism against LDL oxidation. Reaction 4 describes the scavenging of $LOO^{\bullet}$ radicals by antioxidant (AH) yielding LOOH and antioxidant radicals $A^{\bullet}$. The computed data are presented as lines numbered 1, 2, 3 for the indicated experimental groups H, M and L in Fig. 1. The difference between experimental and simulated data expressed in currently accepted parameters (lag time, half time, rate and the extent of oxidation using the same fitting sigmoidal function, see [9]) was about 4% on average.

This study supports some of the earlier observations that cholesterol rich LDL and/or hypercholesterolemia accelerates the initial phase of LDL oxidation [19–21]. This paper shows, in addition, the existence of a negative correlation between the level of LDL cholesterol and antioxidant effect of ascorbic acid. The simulation model indicates that the decreasing antioxidant defence is not directly related to the raising cholesterol content in LDL but rather to its oxidizable component (see LH in Table 1) and to the increasing concentration of preformed lipid hydroperoxides [22,23]. The association between these two variables was observed earlier under various conditions [24–26]. It was also found that preformed lipid hydroperoxides decrease due to administration of antioxidants in vitro as well as in vivo [11,27]. It should be noted that the variable effect described here was not specific for ascorbic acid but it was found also when using other antioxidants of the polyphenolic type (see [28–30]).

This study may explain why in some works the administration of antioxidant failed to stop the development of atherosclerosis especially under the existence of higher cholesterolemia [3,5], because hypercholesterolemia, albeit indirectly increases resistance to the antioxidant effect. However, it remains unclear why in other papers such failure was accompanied by the prolongation of the lag phase of LDL oxidation [4,6]. The simulation model of LDL oxidation with no additional data can offer only few general explanations. It is obvious that atherosclero-

Table 1
Reactions, rate constants and metabolites initial values used by simulating the LDL oxidation

Reaction no.	Rate constants (M^{-1} s^{-1})								
	Group H			Group M			Group L		
	A0	A1	A2	A0	A1	A2	A0	A1	A2
Cu(II)/Cu(I)									
1. $LOOH \rightarrow LOO^{\bullet}$	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1×10^{-7}	1.5×10^{-7}	1×10^{-7}	1×10^{-7}
2. $Lh+LOO^{\bullet} \rightarrow LOOH+L^{\bullet}$	8	8	8	18	19	18	4.8	4.8	4.8
3. $L^{\bullet}+O_2 \rightarrow LOO^{\bullet}$	3×10^{8}	3×10^{8}	3×10^{8}	3×10^{8}	3×10^{8}	3×10^{8}	3×10^{8}	3×10^{8}	3×10^{8}
4. $Ah+LOO^{\bullet} \rightarrow A^{\bullet}+LOO$	920	920	920	920	920	920	920	920	920

The experimentally found constants (see [15]) were slightly modified for best fit to the experimental data of group H, M and L described in the text. Each group comprised three sets A0, A1 and A2 corrresponding to the increasing amount of ascorbic acid added to the LDL samples.

The initial values of LOOH (a.u.) for the sets A0, A1 and A2 in group H were: 0.027, 0.03, 0.033, in group M: 0.002, 0.002, 0.002 and in group L: 0.001, 0.0005, 0.0005, respectively. The values of Lh (a.u.) for all the groups H, M and L were: 0.86, 0.85 and 0.42, respectively. The initial values of radicals $LOO^{\bullet}$, $L^{\bullet}$ and $A^{\bullet}$ were set to zero and the fixed value of oxygen was set to 0.0001 M for all models. For Ah it was in all sets (in M): 1×10^{-6} (assumed amount of endogenous antioxidant), 3.2×10^{-6} and 5.5×10^{-6}.

Abbreviations: LOOH, lipid hydroperoxides; $LOO^{\bullet}$, lipid peroxyl radical; Lh, oxidizable lipid or PUFA; $L^{\bullet}$, free lipid radical; O_2, oxygen; Ah, antioxidant, ascorbic acid; $A^{\bullet}$, radical Ah; Cu(II)/Cu(I), copper-catalyzed reaction; a.u., absorption units.

sis development in vivo is affected by many determinants including the continuous production of oxidizable lipids. Some of them most likely do not exist during the in vitro test for LDL oxidation [31]. The preformed lipid hydroperoxides due to intensive antioxidant therapy could remain low under ex vivo conditions resulting in high resistance of LDL to oxidation, whereas under conditions in vivo other determinants of LDL oxidation could predominate. The simulation model predicts, for example, that the effect of a 10-fold increased concentration of antioxidant could be eliminated by a 3-fold greater rate constant of reaction 1 or, by a 2-fold greater rate constant of reactions 1 and 2.

It should be remembered, in addition, that the relative increase of cholesterolemia experimentally induced is usually much higher and of shorter duration than that in man. Thus, other conditions for atherosclerosis development could be initiated [32–34].

Remembering this, the possible clinical implications include the importance of decreasing the oxidizable lipid substrate and increasing exponentially antioxidant doses to achieve protection against LDL oxidation.

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